BACILLUS PROTEUS VULGARIS

A CLINICAL AND BACTERIOLOGICAL INVESTIGATION

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The work for the present paper was suggested by the frequency with which Bacillus Proteus Vulgaris was found to occur in cases of Chronic Otitis Media and its intra-cranial complications. The organism is usually looked upon as being a purely saprophytic and non-pathogenic one; but numerous cases have been described of its occurrence in suppurative conditions in various parts of the body, some of these being apparently in pure culture.

Hauser[1] first described the group, and isolated three forms which his later observations led him to believe were but variations of the same species.

Ohlmacher[2] found Bacillus Proteus Vulgaris with Staphylococcus Cereus Flavus and Staphylococcus Albus in a Cerebellar abscess following Chronic Otitis Media: and the organism was also found in a case of Meningitis after Chronic Otitis Media.

Wolf[3] describes a case of Pyelonephritis in which the organism was found in pure culture; and a case of cystitis in which it was also present.

Out of 26 cases of mastoid abscess with cerebral

complications Lauffs[4] found the bacillus in 6. In two cases it was in pure culture, in three Streptococci were also present, and in one case there were Streptococci and Diplococci. One of these cases was a cerebellar abscess, three were perisinus abscesses, one a subdural abscess, and one a cerebellar abscess with sinus thrombosis.

Libman and Celler[5] describe the organism in a case of Suppurative Otitis Media with Sinus Thrombosis.

Ross[6] describes a case of Meningitis in which the only organism that could be cultivated from the cerebro-spinal fluid was a bacillus of the Proteus group.

Meldorf[7] found Proteus Vulgaris in two cases of Acute Intestinal Catarrh.

Schnitzer[8] and Krogius[9] found the bacillus in cases of Cystitis.

Flexner[10] isolated it from a case of Peritonitis.

Reed[11] found it in a case of Crupous Pneumonia associated with the Pneumococcus; and Charrin[12] describes it in Pleurisy.

Maleschini[13] found Proteus in a case of Meningitis following Otitis Media.

In the Pathological Department of the Royal Infirmary of Edinburgh during 1910, 1911, and 1912

the bacteriology was investigated in 22 cases of intra-cranial complications of Chronic Otitis Media. In 7 of these Bacillus Proteus Vulgaris was found to be present, and in 6 others there was a Gram-negative bacillus whose nature was not determined.

Logan Turner[14] noted the occurrence of Bacillus Proteus Vulgaris in the clot of a lateral sinus following Chronic Otitis Media, and in the Cerebro-spinal fluid of a meningitis case following Chronic Otitis Media. Cholesteatoma was present in the tympanum of both cases.

Out of 21 cases of Chronic Otitis Media Reynolds[15] found the bacillus in 12.

The same worker found the organism in a cerebellar abscess, in cerebro-spinal fluid, and in two lateral sinus clots: all four cases being complications of Chronic Otitis Media.

Fraser and Dickie[16] noted the presence of the bacillus in 15 out of 33 of their cases in which the bacteriology of Chronic Otitis Media was determined.

In this investigation I took specimens of pus, in some cases from the meatal discharge, in others from the tympanum at operation; and the method of isolation

was as follows:-

Ordinary peptone broth tubes were inoculated with the pus and incubated at 37°C for 24 hours. A very small platinum loopful was then transferred to an agar Petri plate and spread over the surface of three consecutive plates with a glass spreader in the usual way.

It was found essential to dry the surface of the agar before using it. If a tube of agar be poured into a plate and after cooling be inoculated without being dried, Bacillus Proteus Vulgaris if present will rapidly spread in the surface film of moisture and soon cover the whole surface of the plate, owing to its streaming method of growth. Hence it is impossible to separate other organisms from the Proteus; and the plate if hurriedly examined may appear to contain a pure culture of Proteus although in reality it is mixed with other organisms. If however the agar plate before being used is put into a hot air oven, and the lower part of the dish turned over so that it rests with its edge on the lid; and then the contents of the oven be raised to 65° C for 5 or 10 minutes, - the surface of the agar will be found to be quite dry, and after 24 hours' incubation will invariably show isolated colonies of the Proteus organism on the third

plate.

A single colony is taken from the third plate and incubated in broth for 24 hours: this is a "presumably" pure culture. A small loopful is taken from this broth and three consecutive dried plates again inoculated with the spreader, and incubated for 24 hours.

A single colony is again removed from the third plate to broth for 24 hours at 37° C. The plating is then repeated and a single colony transferred to broth for 24 hours. And for still one more time a small loopful is plated on three consecutive dried plates. A single colony from the third plate may be taken as a "proved" pure culture.

Hence to get a "proved" pure culture one must plate out at least three times after the "presumably" pure culture is obtained.

The pus was always put first into broth for 24 hours and not straight on to agar, as it was found by Lewis[14] that in this way one sometimes gets an organism which is missed when the pus is put straight on to agar. Thus a mixture of Bacillus Proteus Vulgaris with a small quantity of another organism may appear to be a pure culture of the Proteus if the culture is made straight on to agar instead of being first put into broth for 24 hours.

Different authors seem to have very different ideas as to what are the cultural and other characters of Bacillus Proteus Vulgaris. The organism which was obtained from the ear invariably gave the following characteristics, and any which did not were discarded so far as this work is concerned.

In size it is very pleomorphic - varying from a long bacillus to a small coccus. It has flagellae: does not form spores: is motile: and is decolourised by Gram's method. It is aerobic and facultatively anaerobic.

On agar it forms a pale, almost colourless colony which does not show any definite granular structure under the microscope. The edge of the colony shows the characteristic "spiking" due to rapid and irregular spread over the surface of the agar.

On a gelatin plate the spiking is very well seen; and the gelatin is rapidly liquefied. A gelatin stab soon shows liquefaction in a saccate manner; and in a few days the whole tube is liquefied.

Broth shows diffuse turbidity without surface pellicle.

On potato there is a luxuriant growth; almost colourless, or dirty white in colour.

Acid and gas are formed in Glucose litmus broth.

Neither acid nor gas is formed in Lactose litmus

broth.

Sulphide of Hydrogen and Indol are formed in cultures.

Milk is coagulated with transient acid production, but the medium rapidly becomes alkaline and the clot is peptonised.

Other characters given by various authors are:- Fermentation of Cane sugar with or without formation of free oxygen (Liborius[18]). Liquefaction of blood serum (Ford[19]). Glen[20] notes that there is no dulling or wrinkling of agar. Smith[21], starting with a pure culture of Bacillus Proteus Vulgaris, by simply replating many times and selecting from the colonies on the plate the one which liquefied most rapidly and the one which liquefied most slowly, obtained a rapidly liquefying culture and one which hardly liquefied at all.

Nitrate is reduced to nitrite, or even to ammonia. The reaction of common media becomes strongly alkaline owing to decomposition[22].

The bacillus is non-chromogenic[23], and non-fluorescent[23]. Glen makes the interesting observation that Lactose is fermented by the organism when it is grown anaerobically[20].

Loghen and Loghen-Pouw[25] describe a form which does not form indol.

Castellani[26] observes that Maltose and Galactose are fermented by Bacillus Proteus Vulgaris with formation of acid and gas. Dulcite, Mannite and Sorbite are unchanged.

Most of the other members of the Proteus group retain the stain by Gram's method. Bacillus Proteus Zenkeri' and Bacillus Proteus Zopfii[27] do not liquefy gelatin. Bacillus Proteus Mirabilis (Hauser) liquefies gelatin slowly but is Gram-positive.

I examined specimens of pus from 58 cases of Chronic Otitis Media, 20 cases of Acute Otitis Media, and 15 cases with presumably healthy ears.

Bacillus Proteus Vulgaris was only found in the chronic cases, and out of the 58 chronic cases the organism was present 24 times.

In those cases where the pus was obtained at operation (Radical Mastoid) the bacillus was only found when there was cholesteatoma present. But on the other hand cholesteatoma was present 11 times when no Proteus was demonstrable.

Of the 24 cases where the organism was found 18 were operation cases and cholesteatoma was present. The other 6 cases where I obtained the organism were

patients under conservative treatment, and hence as no operation was done the presence or absence of cholesteatoma could not be determined.

This constant association of cholesteatoma with the bacillus suggests that the former is a particularly suitable nidus for the growth of the organism. And it probably justifies one in saying that if one finds the bacillus in a meatal discharge then the case will not get better without operation. But it is worthy of note that Reynolds[15] found Bacillus Proteus Vulgaris in 3 cases where at operation no cholesteatoma was present.

In none of the cases was the bacillus present in pure culture. It was usually associated with one or more of - Staphylococcus Aureus, Streptococcus, Pneumococcus, and other organisms.

The absence of Bacillus Proteus from all the cases of Acute Otitis is in accordance with the results of other workers. Reynolds[15] found the bacillus in one out of 15 cases examined: but this patient had had discharge from the ear for 3 months and so (as Reynolds suggests) the case may almost be looked upon as chronic one.

Fraser and Dickie[16] note the absence of the organism in 18 Acute Otitis cases.

I also examined specimens from 6 cases of intra-cranial complications following Chronic Suppurative Otitis Media. In two of these (Cerebellar Abscess, and Basal Meningitis) no Proteus organism was found. But in the other four cases Bacillus Proteus Vulgaris was obtained: they were - Lateral Sinus thrombosis (2 cases) in which the organism was present in the clot; Temporo-sphenoidal abscessm in which it was present in the abscess; and Extra-dural abscess, in which it was also found.

But in none of the cases was the organism in pute culture.

It seems possible that those observers who report pure cultures of the bacillus from intra-cranial and other cases may really have been dealing with a mixed infection; the mistake having arisen owing to the capacity which the organism possesses of rapidly spreading over the surface of a damp agar plate, and so masking the presence of anything else which may happen to be there.

The literature on the agglutination of Bacillus Proteus Vulgaris is rather confusing and contradictory.

Pfaundler[28], Grassberger[29], Jochmann[30], and Klieneberger[31], found high agglutination in the serum of patients for the homologous strain, so that they considered the causal relationship of the bacillus was certain.

Lannelongue and Achard[32] obtained an agglutination reaction with the serum from animals infected with Bacillus Proteus. This was most marked with the strain infecting the animal, but also affected other strains.

In his case of Pyelonephritis Wolf[3] obtained agglutination with a dilution of 1 in 100. And in a case of Cystitis in a dilution of 1 in 500.

Klieneberger[31] found that a Proteus Vulgaris immune serum agglutinated equally all strains, and hence the stains are closely allied.

According to Wolf[3] however Bacillus Proteus agglutinates like Bacillus Coli, and so the reaction is of no use for diagnosis.

Rodella[33] found that the agglutination reaction gave varied results, owing, he thinks, to different organisms being included under the name.

Cross agglutination on Bacillus Typhosus in cases of Proteus infection has been often observed (Lubowski and Steinberg[34]; Jochmann[30].)

In Ross' case of Meningitis[6] with a pure culture of

the bacillus in the cerebro-spinal fluid, the serum of the patient agglutinated the bacillus in dilutions up to 1 in 30 only.

In 10 cases I performed an agglutination reaction. This was done by the macroscopic method in the following manner:-

Ordinary test-tubes are taken, and into the first is put 1.8 c.c. of physiological saline, and then 0.2 c.c. of serum added. This gives a dilution of 1 in 10. The fluids are thoroughly mixed together, and then 1 c.c. is removed to the next tube and 1 c.c. of saline added: thus a dilution of 1 in 20. After mixing, 1 c.c. is transferred to the next tube and 1.5 c.c. of saline added: thus a dilution of 1 in 50. Half a c.c. from this tube is discarded, and 1 c.c. taken on to the next tube and an equal volume of saline added. Hence a dilution of 1 in 100. And so on until the required dilution is obtained. Each tube thus contains 1 c.c. of fluid: and a control tube containing 1 c.c. of normal saline is also used.

One platinum loopful of a 24 hour old agar culture is then added to each tube, being emulsified first on the side of the tube.

If the reaction is positive "clumping" is seen

after an hour at 37° C. That is to say, the emulsion instead of being uniformly turbid shows little specks scattered through it.

A strongly positive reaction is seen in a few minutes at room temperature.

In the 10 cases in which the agglutination reaction was performed each patient's serum was used to agglutinate the strain of Proteus isolated from himself. But in no case was a positive reaction obtained.

In 4 cases there was no agglutination with a dilution of 1 in 10. Two showed agglutination with 1 in 10 but not with 1 in 20. Three agglutinated 1 in 20 but not 1 in 30. And one showed clumping in a dilution of 1 in 30 but not in higher dilutions.

One of the 10 cases was that of an Extra-dural abscess in which the bacillus was found accompanied by Staphylococcus Aureus and Streptococcus. But the patient's serum did not agglutinate his own strain even in a dilution of 1 in 10.

An agglutination with a dilution of 1 in 30 cannot be called a positive reaction, for I have obtained agglutination up to 1 in 20 with normal human serum.

I then made experiments to determine whether or not a definite method of diagnosis of the identity of the bacillus could be obtained by serological means. There are three serological proceedings which are in general use for this purpose, namely the Agglutination reaction, Complement deviation, and the Precipitin reaction. And if one of these proves to be specific for a micro-organism then the identity of the organism can at any time be determined absolutely.

To carry out these experiments specific sera are necessary; these were obtained in the following way:-

Rabbits were injected, each with 6 doses of a Proteus Vulgaris strain at intervals of about a week. The injections were prepared by growing the strain on agar for 24 hours, then emulsifying in physiological saline, and killing the organism by heating to 60°C for 1 hour. The first dose was the surface growth from one agar slope; the second dose from 2 agar slopes; the third and fourth from 2 agar plates; and the fifth and sixth from 4 agar plates.

All the injections were made subcutaneously.

After the 6 injections a clear week was allowed to elapse, and then the rabbits were bled and the serum separated and filtered.

Three rabbits were treated in this way with three strains of Bacillus Proteus Vulgaris, - one from an ear (Strain 3), one from an intestine (Strain 5), and one from a nose (Strain 10). And the homologous sera obtained are called respectively Serum3, Serum 5, and Serum 10.

It was found that Serum 3 agglutinated Strain 3 up to dilutions of 1 in 5000 quite distinctly.

Serum 5 agglutinated Strain 5 up to dilutions of 1 in 500.
Serum 10 - - 10 - - - of 1 in 2000.

But on doing cross agglutinations it was found that Serum 3 only agglutinated Strain 5 and Strain 10 up to dilutions of 1 in 20. Serum 5 agglutinated Strain 3 up to dilutions of 1 in 20, and Strain 10 in dilutions of 1 in 50.

Serum 10 agglutinated Strain 3 in dilutions of 1 in 10 only, and Strain 5 in dilutions of 1 in 20;

(SEE TABLE)

	Serum 3 & Strain 3	Serum 3 & Strain 5	Serum 3 & Strain 10
1 in	10 +++	+++	+++
"	20 +++	+	+
"	50 +++	-	-
"	100 +++	-	-
"	200 +++	-	-
"	500 +++	-	-
"	1000 +++	-	-
"	2000 +++	-	-
"	5000 +	-	-
Control	-	-	-

+++ indicates complete agglutination.
+ " partial "
- " no "

	Serum 5 & Strain 5	Serum 5 & Strain 3	Serum 5 & Strain 10
1 in	10 +++	+++	+++
"	20 +++	+	+++
"	50 +++	-	+
"	100 +++	-	-
"	200 +++	-	-
"	500 +	-	-
"	1000 -	-	-
Control	-	-	-

	Serum 10 & Strain 10	Serum 10 & Strain 3	Serum 10 & Strain 5
1 in	10 +++	+	+++
"	20 +++	-	+
"	50 +++	-	-
"	100 +++	-	-
"	200 +++	-	-
"	500 +++	-	-
"	1000 +++	-	-
"	2000 +	-	-
"	5000 -	-	-
Control	-	-	-

Control experiments done with normal rabbit's serum showed no trace of agglutination with 1 in 10 dilutions with either Strain 3, Strain 5, or Strain 10.

These results were confirmed by agglutination experiments with the three specific sera acting on three other strains of Proteus - Strains 7, 11, and 15, - all from the ear. No agglutination was obtainable with dilutions greater than 1 in 50.

	Serum 3 & Strain 7	Serum 3 & Strain 11	Serum 3 & Strain 15	Normal serum & Strain 7.
1 in 10	+++	-	-	+
" 20	+	-	-	-
" 50	-	-	-	-
Control	-	-	-	-

	Serum 5 & Strain 7	Serum 5 & Strain 11	Serum 5 & Strain 15	Normal serum & Strain 15	Normal serum & Strain 11
1 in 10	+	-	+	-	-
" 20	-	-	-	-	-
" 50	-	-	-	-	-
Control	-	-	-	-	-

	Serum 10 & Strain 7	Serum 10 & Strain 11	Serum 10 & Strain 15
1 in 10	+++	-	+++
" 20	+	-	+
" 50	-	-	+
" 100	-	-	-
Control	-	-	-

Hence the Agglutination reaction cannot be used as a means of diagnosis of Bacillus Proteus Vulgaris. For strains which appear to be identical in their cultural, morphological, and staining characteristics may give very different agglutination results when acted upon by a Proteus immune serum. (Compare Klieneberger's results given above).

To find whether one strain could absorb the agglutinin obtained by injection of another strain, I then did the following experiment. To a 1 in 10 dilution of Serum 3 an excess of Strain 10 was added and the resulting mixture placed in the incubator at 37° C for one hour. The fluid was then centrifuged and the clear portion pipetted off; and an agglutination reaction done with this and Strain 3.

A similar experiment was done by adding excess of Strain 3 to Serum 10, and then agglutinating Strain 10 with the resulting fluid.

It was found in both cases that the "foreign" strain did not absorb any of the agglutinin belonging to the homologous strain: so that a serum after treatment with another strain agglutinated its own strain just as well as an untreated serum.

	Serum 3 & Strain 3.	Serum 3, saturated with excess of Strain 10, & Strain 3.
1 in	10	+++	+++
"	20	+++	+++
"	50	+++	+++
"	100	+++	+++
"	200	+++	+++
"	500	+++	+++
"	1000	+	+
"	2000	+	+
"	5000	-	-
Control		-	-

	Serum 10 & Strain 10	Serum 10, saturated with excess of Strain 3. & Strain 10.
1 in	10	+++	+++
"	20	+++	+++
"	50	+++	+++
"	100	+++	+++
"	200	+++	+++
"	500	+++	+++
"	1000	+	+
"	2000	+	+
"	5000	-	-
Control		-	-

Hence the absorption of agglutinin is of no value as a reaction for diagnosis of the identity of the bacillus.

I found that Serum 3 agglutinated the Typhoid bacillus up to dilutions of 1 in 100. Typhoid immune serum did not agglutinate Strain 3 in dilutions of 1 in 10.

Excess of Strain 3 was then added to Serum 3, the mixture kept at 37°C for one hour, then centrifuged and the clear fluid used to agglutinate Bacillus Typhosus. It was found that no agglutination was produced with dilution of 1 in 10. Hence saturation of a Proteus immune serum with the homologous strain causes absorption of all the agglutinin for Bacillus Typhosus.

	Serum 3 & Bac. Typhosus.	Anti-typhoid serum & Strain 3.
1 in 10	+++	-
" 20	+++	-
" 50	+++	-
" 100	+	-
" 200	-	-
Control	-	-

Serum 3, saturated with excess of Strain 3. & Bac. Typhosus.

1 in 10	-
" 20	-
Control	-

Similarly saturation of a Proteus immune serum with

Bacillus Typhosus causes absorption of part, but not all, of the agglutinin for the homologous Proteus strain.

	Serum 3 & Strain 3	Serum 3, saturated with excess of Bacillus Typhosus & Strain 3.
1 in 10	+++	+++
" 20	+++	+++
" 50	+++	+++
" 100	+++	+++
" 200	+++	+
" 500	+++	+
" 1000	+++	-
" 2000	+	-
" 5000	-	-
Control	-	-

As the Agglutination reaction failed to give a satisfactory means of diagnosis of the identity of Bacillus Proteus Vulgaris, the second great serological reaction was tried for this purpose - namely the Complement Deviation reaction.

The antigen for these experiments was obtained by growing each strain on three agar plates for 24 hours at 37°C. Two c.c. of distilled water were then added to each plate and an emulsion made. The emulsion was shaken for 12 hours, centrifuged, the extract pipetted off and carbolic acid added - one part of 1 in 20 acid to 9 parts of extract.

From these experiments it appeared that the complement-deviating substance is not specific and different for each strain as is the agglutinating substance: but that working with one antibody (Serum 3), and three antigens (Extracts 3, 5, & 10), there is deviation in each case. No deviation was obtained with extract of Bacillus Typhosus.

But the results were not absolutely definite as the specific sera were not strong enough in complement-deviating substance to perform very conclusive tests.

This line of work was not proceeded with however as precipitin experiments seemed to hold out a better prospect of success.

The third great serological test was then proceeded with, namely the precipitin reaction.

In these experiments a strain of Bacillus Proteus Vulgaris is grown for a certain time in broth - the length of time depending on the conditions: and the broth is then filtered through a Berkefeldt filter. The filtrate is then added to a Proteus-immune serum in a narrow glass tube, and this is placed in an incubator at 37° C. After some time a cloudiness appears in the solution; this gets more marked, and flakes appear which gradually fall to the bottom as a

precipitum.

The length of time which the precipitum takes to appear varies with the serum and the filtrate, but as a rule some cloudiness can be seen after an hour, and the precipitum has completely formed in 24 hours.

This reaction was tried in 10 cases of Bacillus Proteus Vulgaris, and in every case a well marked precipitum was obtained.

So that this reaction constitutes itself a test by which the identity of Bacillus Proteus Vulgaris can at once be proved by serological methods.

The Precipitum reaction is however only quantitatively and not qualitatively specific.

Kraus[35] first described the reaction and thought it was absolutely specific.

Later Wassermann[36] and Tupnix[37] showed that the specificity is quantitative rather than qualitative.

Norris[38] concludes that "no precipitating relationship exists between Bacillus Proteus and the bacilli of the Coli-typhoid group or the pyogenic cocci, but that the Spirillum of Metchnikof may give a reaction".

In view of this statement I tried the filtrate of a

Spirillum of Metchnikof but no precipitum formed.

Filtrates of Bacillus Coli, Typhosus, Subtilis, Diphtheria, Actinomyces and Mallei, also failed to give any precipitin. Bacillus Pyocyaneus gave a cloudiness but no definite precipitum.

The proportional amounts of filtrate and serum required to form a good precipitum seem to vary with the strength of the filtrate and the immune strength of the serum: so that no hard and fast rule can be laid down. In general I found however that equal quantities of filtrate and serum gave a good reaction.

Norris[38] used $\frac{1}{4}$ c.c. of serum to 1 c.c. of filtrate. But it should be pointed out that, as was noticed by Halban and Landsteiner[40] and by Eisenberg[41], the precipitum formed is relatively soluble in excess of filtrate.

The method adopted in preparing the bacterial filtrate has varied with different workers.

Kraus[35] grew his organisms, exposed them to a pressure of 300 atmospheres, and then filtered.

Morris[38] and Gaehtens[39] incubated for several months before filtering.

I found that by growing a Proteus strain for a month in broth and filtering, a slight precipitum

could be obtained with Proteus immune serum. If however this broth culture were shaken for 12 hours before filtering, this precipitum was distinctly more marked. This result and the fact that Kraus obtained a better precipitum after exposing his broth to a pressure of 300 atmospheres, suggested that oxygenation of the broth culture before filtering is necessary for the production of a good precipitum. Hence the following experiment was tried:-

Strain 3 was grown in broth in a flat-bottomed toxin flask for ten days, so that the culture had free access of air, and the flask was gently shaken each day. After the ten days it was filtered and the filtrate was found to give a well marked precipitum with Serum 3.

The same strain was grown in similar broth in a narrow necked flask filled right up to the neck, so that there was very limited access of air; and after ten days was filtered. The filtrate gave no trace of precipitum - not even a cloudiness - with Serum 3!

I found also that bubbling oxygen through a broth culture caused the production of even a better precipitum than shaking for a similar period (24 hours).

Hence the essential for a good filtrate is free access of oxygen; and probably Kraus' pressure of 300 atmospheres, and Norris' and Gaehtens' lengthy

incubation, act by virtue of the oxygenation produced.

As already mentioned the precipitin reaction is considered to be only quantitatively specific, but the results of oxidation suggest that by an elaboration of such treatment the reaction may be made absolutely specific.

With regard to the relationship of Agglutinin to Precipitin the most contradictory statements have been made.

Beljajew[42] thinks there is no parallel between the formation of agglutinins and precipitins. For the formation of precipitins he finds a longer immunisation is necessary.

Gaehtens[39] too thinks that agglutinin and precipitin are not the same, but contrary to Beljajew he finds that precipitin appears in the serum before agglutinin.

On the other hand Fukuhara[43] found equal production of agglutinin and precipitin and considers them to have a very distinct relationship to one another.

And Basil, Oskar, and Tsuda[44] think that bacteriolysin, agglutinin, and precipitin are identical.

But I have shown above that each strain of Bacillus Proteus Vulgaris possesses a separate and distinct agglutinin, while all the strains appear to have one

common precipitin. So that precipitin and agglutinin cannot have any very definite relationship to one another.

SUMMARY.

Isolation of Bacillus Proteus Vulgaris can only be accomplished by using specially dried agar plates.

The bacillus only occurs in the chronic forms of Otitis Media, and it is almost invariably associated with the presence of Cholesteatoma.

The organism rarely, if ever, occurs in pure culture in the ear.

Under ordinary circumstances the bacillus plays a passive part in Chronic Otitis Media; but with the lighting up of an active intra-cranial complication it may become pathogenic.

The agglutination reaction cannot be used for the recognition of Bacillus Proteus Vulgaris in general, for each strain possesses a separate and distinct agglutinin.

The precipitation reaction can however be used for proving the identity of the bacillus by serological means.

For the preparation of a useful filtrate for the

precipitation reaction free access of oxygen (air) is necessary during growth.

There is no definite relationship between agglutinin and precipitin.

In conclusion I would like to thank Dr. Logan Turner for the facility with which I was able to obtain material: and Professor Lorrain Smith and Dr. F. E. Reynolds for their advice and help in the work.

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